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Optimization Process by Complex Coacervation of Fish Oil using Gelatin/ SDS/ NaCMC and Secondary Coating Application with Sodium Polyphosphate.

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Abstract

Microencapsulation of fish oil was investigated using gelatin/SDS/NaCMC as the coating materials. The microencapsulation efficiency (EE) of microencapsulated fish oil (MFO) was investigated with respect to three variables including the Concentration of Wall Material (CWM), Ratio of Core and Wall Material (RCW) and the pH. The response surface model for microencapsulation efficiency showed a high coefficient of determination and a non-significant lack of fit ($p < 0.05$). The optimum microencapsulation efficiencies were 75.2 ± 0.73% and 76.86 ± 0.46% for microcapsules have one shell (MFO1) and multishells (MFO2) during spray drying respectively and 53.2 ± 0.39% and 56.63 ± 0.73 % for microcapsules have one shell and multishells during freeze drying, respectively. Compared to MFO, the peroxide value (POV) of the total oil from the MFO under the optimized conditions was significantly lower at 60 ± 1 °C after 30 days, which indicates a promising feature as functional food ingredients.

Keywords: Complex coacervation; Response surface methodology; PUFA; Lipid oxidation.

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1. Introduction

Functional foods containing omega-3 lipids is one of the fastest growing food product categories with around 1300 omega-3 fatty acids enriched products launched in 2007 in the USA and Europe [4]. The current intake of omega-3 fatty acids in a typical Western diet is lower than the recommended level and the intake of PUFAs consists primarily of omega-6 fatty acids [1, 2]. The present Western diet is estimated to have 10 to15 times higher intake of omega-6 than omega-3 fatty acids [2]. The low intake of omega-3 fatty acids and increasing scientific evidence of the beneficial effects of EPA and DHA has led to introduction of omega-3 fatty acids enriched foods in the market [3].

Microencapsulation by coacervation has been defined by Gouin as the phase separation of one or many hydrocolloids from the initial solution and the subsequent deposition of the newly-formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media [6]. Not all polyelectrolytes exhibit this phenomenon, which depends on several conditions such as pH, charge density on the polymers, colloid concentration, ionic strength of the medium, temperature, etc [5];The microencapsulation technology has been used to encapsulate flavor oil [7-9], vitamins [10], casein hydrolysate [11], and drugs [12] to retard or avoid the oxidation of these oils has been reported and it has drawn considerable attention in the food industry [13-17, 18, 19].

Gelatin, a polyampholyte and one of the most important wall materials in microcapsule production. Studies were performed on the protein/polysaccharide systems, mainly gelatin/gum arabic. And the formation of the microcapsules was attributed to the interaction between protonated amino groups of the protein and deprotonated carboxyl group of the polysaccharide.

Sodium carboxymethylcellulose (NaCMC), a derivative of cellulose, is an anionic polyelectrolyte which can easily ionize. Guillot [20] reported that NaCMC has no surface activity at concentrations below 7 g/L and can hardly be adsorbed at oil/water interface. Li [21]enhanced the adsorption of NaCMC on the oil/water interface by adding appropriate amount of sodium dodecyl sulfate (SDS) into the low concentration solution of NaCMC, and prepared the optical transparent microcapsules by mixing the gelatin to NaCMC and SDS as the capsule wall.

In this paper, we have studied for the first time, the effect of having multiple shells in the microencapsulation process by spray-drying and freeze-drying of fish oil with gelatin/SDS/NaCMC. Furthermore, oxidative stability (OSI) has been studied. during the microencapsulation process in order to evaluate the fish oil attitude to the spray-drying and freeze drying processing. Different aspect of microencapsulation process such as wall material composition, wall–core micropasules ratio, microencapsulation efficiency (EE) were as well evaluated, and a suitable system for microencapsulation has been achieved.

2. Materials and methods

2.1. Materials

A type gelatin, sodium carboxymethylcellulose, sodium phosphate were purchased from Sinopharm Chemical Reagent, Shanghai Chemical Reagent Corporation (Shanghai, China). Commercial microbial transglutaminase (TG) was obtained from Yiming Biological Products Company (Taixing, China), with a nominal enzymatic activity of 60 U/g, measured by hydroxamate method. Fish oil was granted from Wuxi Xunda Chemicals Company. Sodium hydroxide and acetic acid were purchased from Sinopharm Chemical Reagent, Shanghai Chemical Reagent Corporation (Shanghai, China).

2.2. Methods

2.2.1. Microencapsulation process

One suitable process of microencapsulation using complex coacervation comprises three steps: (1) dispersing the loading substance into a system of at least one of the polymers for the complex coacervate; (2) forming shells by deposition of coacervates which derive from the polymeric components under controlled conditions of temperature, pH, concentration of colloids, mixing speed etc.; (3) and hardening of the shells by crosslinking of the coacervates deposited on microcapsules [22, 23]. The first stage to achieve oil microencapsulation was the formation of a fine and stable emulsion of the core material (oil) in the wall solution. Different ratios oil and wall material were evaluated for each studied wall materials.

2.2.2. Optimization of fish oil microencapsulation process

2.2.2.1. Designs of single factor experiments

Single factor experiments were performed to determine optimal conditions for single factors by analyzing their influences to microencapsulation effect, which were concentration of wall materials (CWM) (gelatin/NaCMC, ratio 9:1), ratio of core material to wall (RCW) and pH value. We have used Design Expert for studying CWM effects on encapsulation, wall materials were prepared into 0.5% (w/w), 1.0% (w/w), 1.5% (w/w), 2.0% (w/w), 2.5% (w/w) solutions, respectively. The pH range was around 4.4 to 4.8.

2.2.2.2. Preparation of emulsion

Step 1: One-layer Gelatin-NaCMC

Fish oil (3 g) was mixed into 150ml of gelatin solution at 50C using a three-bladed Teflon overhead stirrer. It was allowed to stir for 30min for stabilization of oil droplets; 150ml of NaCMC solution (also at 50℃) was then added to the above solution in the ratio 9:1. The mixture was stirred at 12000 rpm for 3 min using a homogenizer (FJ 200-S, Shanghai Specimen Model factory, China). Fish oil was added to form an emulsion and the homogenization was maintained at this rate for 3 min. This level of mixing was found to be sufficient, confirming the findings of Chen [24]as the emulsion was stable even after a month (data not shown). Using 10 % (w/w) aqueous acetic acid, the pH of the emulsion was adjusted to 4.4–4.8 at the stirring speed of 400 rpm by overhead stirrer (RW 20 digital n, IKA, Germany). After 15 min, the system was then slowly allowed to cool to room temperature. Formation of coacervate capsules was followed by observing samples under an optical microscope. The rate of cooling was found to be crucial for successful encapsulation and should not be more than 1℃ per min. Once the system attained room temperature, it was cooled, using an external ice bath, to 10℃.

The nascent capsules were then cross-linked by adjusting the pH to 6.0 while transglutaminase (15U/g gelatin) was added to harden the microcapsule walls under agitation during 12 hours.

Step 2: Second shell: Gelatin – Sodium polyphosphate

A gelatin solution and sodium polyphosphate were prepared at a concentration rate of 9:1; The gelatin and polyphosphate solutions were combined to form a mixture, and pH of the mixture was adjusted to 4.7 with 10% aqueous acetic acid. The mixture from Step 2 was added to the mixture with coacervates formed in step 1(Before cross linking). Cooling was carried out under agitation to cause the gelatine and sodium polyphosphate to form coacervates and to coat the lumps formed in Step 1; then transglutaminase was added into the mixture to further strengthen the shell at pH closed to 6.0. After 12 h of hardening, the capsules were then collected by filtration and washed free of excess colloids and cross linker with distilled water; it was then dried.

2.2.3. Emulsion Morphology

The coacervate-rich lower layer was visualized as a function of homogenization rates and total biopolymer concentration. The morphology of moist microcapsules suspended in the water was observed under a light microscope (OLYMPUS BX 51) under an objective magnification of 20x equipped with a camera.

2.2.4. Spray-drying

The emulsions prepared after 12 h of hardening, the microcapsule suspension was fed to a spray drier equipped with a centrifugal wheel atomizer (QZ-5, Wuxi linzhou drying equipment Co. Ltd., china) to get dried coacervate microcapsules [25], operating at an inlet and outlet air temperature of 190 ± 3℃ and 90 ± 3 ℃ and an evaporative capacity of 6 kg/h.

2.2.5. Freeze drying

The freeze-dried microcapsules were produced according to the protocol described by Parris **[26]** with modifications using a lyophilizer (LGJ-10 Freeze Dryer, Beijing, CHINA). The freeze-dried microcapsules were recovered and stored at -18 ℃ until further analysis.

2.2.6. Water activity

The water activity of each sample (500 mg) was measured by an FA-st lab system (GBX WATER ACTIVITY METER,Romans, FRANCE). Each analysis was repeated three times.

2.2.7. Moisture content

The moisture content of each sample (500 mg) was determined by loss of weight in an oven (DHG – 9076A, Shanghai, CHINA) at 105℃; each analysis was repeated three times.

2.2.8. Size distribution of microcapsules.

The analysis of powder particle size was performed using the laser light scattering method by an analyser with a batch cell unit (Mastersizer E, Malvern Instruments, UK). Encapsulated powders were dispersed in propan-2-ol for the particle size analysis and then assayed for size distribution by Mastersizer 2000 laser particle analyzer.

2.2.9. Particle yield

The particle yield for each experimental assay was calculated as reported by Zhong [27].

2.2.10. Microencapsulation efficiency (MEE)

The microencapsulation efficiency is defined as the ratio of core material in the final dried microcapsules to that in the original emulsion [28] as provided elsewhere [13, 17, 29, 30].

2.2.10.1. Total oil

The method described by Wanasundara [31] was used to determine the microencapsulation efficiency of MMO and MSO. The total oil (TO) (%) of microencapsulated fish oils contained both encapsulated and surface oils.

2.2.10.2. Surface oil

The surface oil, also known as the non-encapsulated oil fraction, was determined according to Velasco [17] with modifications.

2.2.11. Flowing properties

The bulk (ρB) and tapped (ρT) densities were determined in a 25 ml glass graduated cylinder as described by Chinta **[32].** The powder flowability was evaluated using the Carr's Index or "percent compressibility" (C) and the Hausner Ratio (HR) [33].

2.2.12. Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids, Color and Moisture of microcapsules

The peroxide value (PV), anisidine value (AV), and free fatty acids (FFA) were determined following American Oil Chemists Society (AOCS) Official Methods [34]. The TOTOX values were calculated as described by Wai [35]. Free fatty acids were measured in PFO, and MFO as described by AOCS method Ca 5a-40 [36], with slight modifications.

2.2.13. Hygroscopicity

For hygroscopicity determination, we use the method described by Cai with little modifications [37].

2.2.14. Scanning electron microscopy

The SEM analysis of the microspheres was carried out by using S-4800 scanning electronic microscope (Hitachi Japan). The samples were sprinkled on one side of double-side adhesive stuck on the stub and then was coated with gold. The microspheres were observed at an accelerating voltage of 10 kV.

2.2.15. Thermal behavior

The thermal behavior was determined by differential scanning calorimetry (DSC) according to Mendanha [11], using a DSC perkinEImer device controlled by a DSC PERKINEIMER (Pyris 1 DSC, USA). The dried samples were added into the DSC sample box with the blank as reference (pans are sealed), and heated from 25 ℃ to 110 ℃ at the rate of 5 ℃/min.

2.2.16. Infrared atlas analysis of microcapsules and wall materials

Bromide potassium was grinded with gelatin, NaCMC, sodium polyphosphates and dried products, respectively, to obtain different powders. The plates were scanned by infrared instruments (FT-IR SPECTROMETER, NICOLET NEXUS).

2.2.17. Determination of Fatty Acid Profile

Fatty acid methyl esters were measured by GC in MFO at day 1 and week 4. Contents of FAME, expressed as a percentage of total FAME measured, in MFO was determined using standard GC methods. Lipid was extracted from microcapsules samples using a modified Folch extraction method.

2.2.18. The storage stability of microcapsules

The prepared microcapsules were firstly put in a desiccator for 48 h and then sealed in hyaline plastic bags and kept in different thermal conditions. The content of fish oil in microcapsules was tested each week. The retention percentage, which was defined as the ratio between the content of fish oil that retained in the microcapsule after some time and the original rate of fish oil in the microencapsulate, was used to evaluate the storage stability of fish oil microcapsules.

Statistical Analysis

The entire experiment was replicated three times and means and standard deviations were reported. Statistical Package for the Social Sciences (SPSS Version 19,IBM) software was used to conduct analyses of variance to determine differences among treatment mean.

3. Results and discussion

3.1. Process optimization

The experiments were arranged according to Box-Benhnken's central composite design. The single factor experiments were as follows: CWM 0.5 - 1.5%, RCW 1:2 - 2:1 and pH value 4.4-4.8. On the basis of these results, taking encapsulation efficiency (EE) as response value of CWM (A), RCW (B) and pH value (C).

The experimental results were analyzed by Design the expert 8.0, the regression equation is:

$$EE\ (\%) = 0.75 - 3.125E\text{-}003A - 5.000E\text{-}003B + 0.028C - 0.041AB - 4.750E\text{-}003AC + 0.017BC - 0.045A^2 - 0.041B^2 - 0.14C^2$$

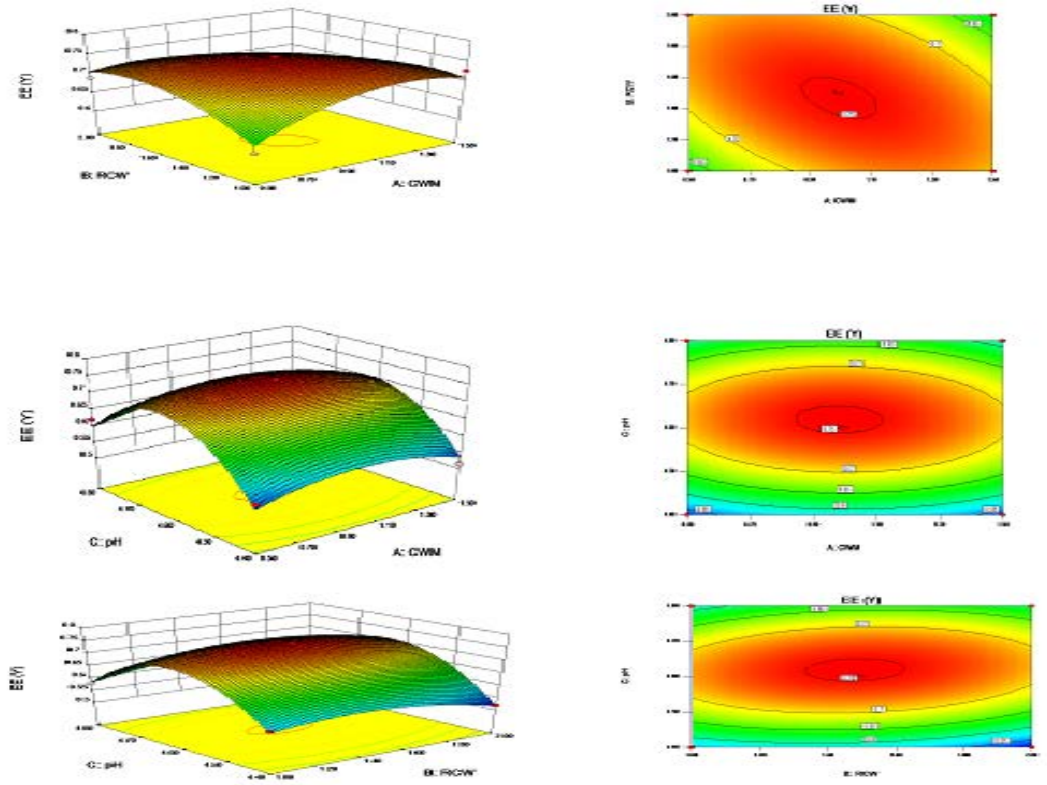

Fig. 1. The contour plots of response surface methodology. (a) The response surface and contour plot of the effect of the wall material concentration and wall core/material material ratio; (b) the response surface and contour plot of the effect of the wall material concentration and the pH value; (c) the response surface and contour plot of the effect of the wall core/material material ratio and the pH value.

3.2. Influence of surfactant

In order to improve the encapsulation efficiency and yield of the process, we studied the effect of adding an oppositely charged surfactant in GE solution at pH 4.6 at 45°C. The figure 2.1 shows the amount of NaCMC adsorbed on oil/water interface increases with the addition of SDS. Further increase of SDS concentration from 1.3 to 2.5mM leads to the decrease of the yield. At 2.5mM, the yield decreases dramatically. [21, 38, 39]. The low yield of the microcapsules could be attributed to the weak interaction between gelatin and NaCMC. It is reported that adding small molecular surfactant into the gelatin and NaCMC system could improve the adsorption of NaCMC on the oil/water interface.[21, 40, 41]

There is a maximum in the yield at 1.3mM, after which the yield decreases rapidly. This is in agreement with results using sodium dodecylbenzyl sulfonate. [40, 42] and with dioctyl sulfosuccinate sodium [41].

In order to understand the interactions between gelatin and SDS we studied the surface tension of the system using a pendant drop apparatus (Dataphysics). According to the results, as shown in Fig 2.2, Gelatin concentration was maintained at 1% by weight, and the SDS concentration was varied from 0.01 to 100mM. The pH was maintained at 4.6 and the temperature at 45°C. These results are qualitatively similar to the results of Knox et al. [43, 44].

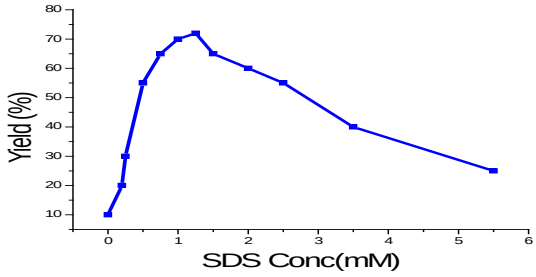

Fig 2.1 Effect of concentration of SDS on microencapsulation yield.

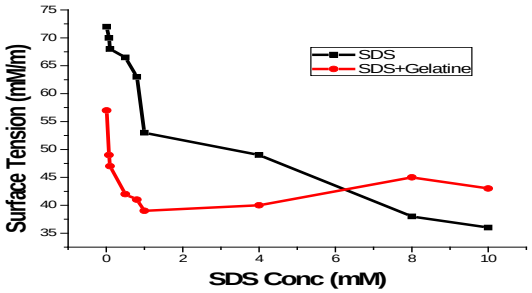

Figure 2.2 Surface Tension of Gelatin-SDS System

The surface tension of pure SDS solutions starts decreasing from 0.05mM and reaches 42 mNm^{-1} at around 8mM, which corresponds to the Sodium CMC. On addition of gelatin, there is a lowering of surface tension to 58 mNm^{-1} for SDS concentrations as low as 0.01mM. This indicates complex formation between SDS and GE at the surface which has a strong surface activity. The surface tension decreases with increasing SDS up to about 0.2mM, after which it remains constant. This can be taken as the critical aggregation concentration (CAC) [45-47]. Above this concentration, complexes are formed mainly in the bulk and are found to be insoluble in water, though they are formed of SDS and GE, which are soluble. [48]Surface tension starts decreasing again only after 50mM, signifying the completion of complex formation, so that surfactant molecules can come to the surface.

3.3. Particle size distribution of microcapsules

According to the result got from MASTERSIZER, it is shown that the microcapsules are spherical in shape and optical transparent. It gives the size distribution of microcapsules prepared with different SDS. It is found that under the same stirring speed, the size distribution of the microcapsules becomes narrow and the average diameter descends with increasing the concentration of SDS. The broad distribution in the size range had the biggest distributional proportion in the range of 10-20 mm, indicating homogeneous microcapsules produced under the optimum condition. Their mean diameter was 8.18 mm for one shell microcapsules and 12.198 mm for multishells microcapsules.

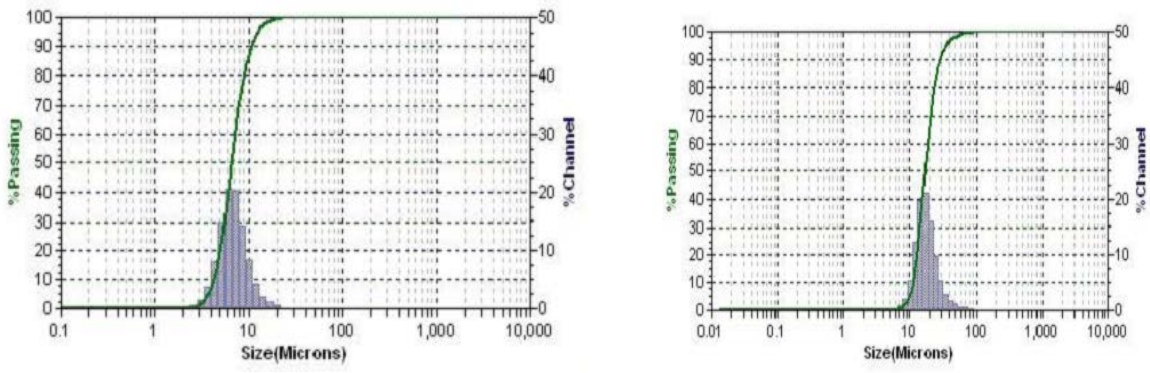

Figure 3. Particle size and particle-size distribution of Microcapsules.

Zeta Potential

Complex coacervation is a pH-sensitive process, because the charge and charge density of polymers vary with pH. The effects of pH on production of the NC were investigated. NC yields were highest at pH = 4.6 and then dropped with the increasing pH. The pH of maximum coacervate yield is believed to correspond to the electrical equivalence pH (EPP), where both polymers carry equal but opposite charges [49]. At the EPP, attracting forces between the charged components neutralize each other, leading to strong binding and the highest coacervation yield.

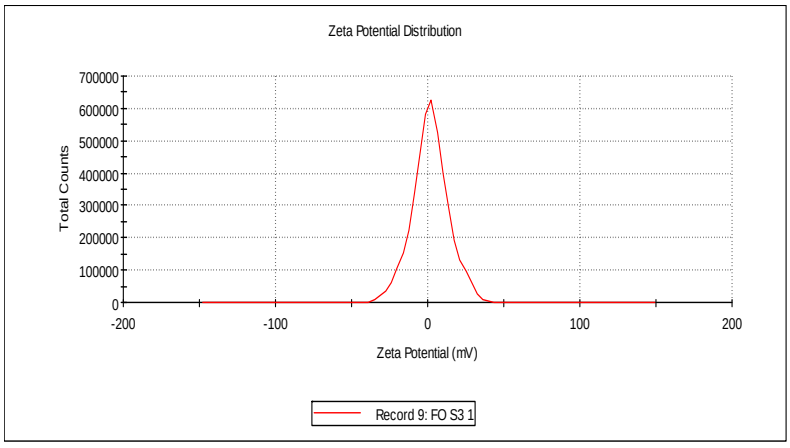

Figure 4. Zeta potential of emulsion at pH=4.6

3.4. Scanning electron microscopy

SEM images of the fish oil microcapsules after spray and freeze drying are shown in figure 5.

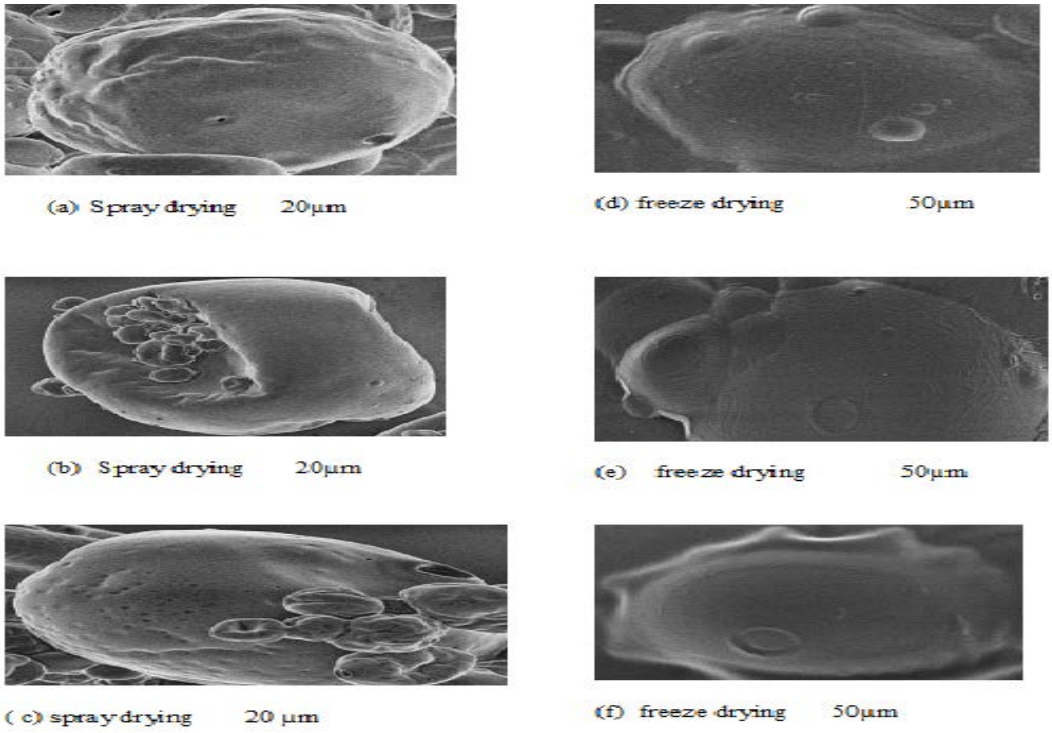

Fig. 5. Differential Scanning electron (a) microcapsules of Gelatin and NaCMC (b) Multi shells

The surface morphology of microcapsules showed that the particles are irregularly shaped with an internal porous network Fig. 5. This observation is similar to that presented by Zhong, Jin, Davidson, and Zivanovic [50]. Microcapsules prepared by freeze drying resulted in small spheres. Furthermore, the particles appear to be agglomerated microcapsules. This behavior was also observed by Parris [26]for the microencapsulation of essential oils with zein by freeze drying. In the microencapsulation of marine oil with alginate/starch blends, Tan, Chan, and Heng [51]observed that microspheres with excessive amounts of surface oil promoted significant agglomeration with reduced microencapsulation efficiency. It is reported that NaCMC/SDS complexes formed due to hydrophobic interaction between NaCMC and SDS help to increase the charge density and amount of polysaccharide in coacervate droplets [21]. Moreover, SDS can interact with positively charged gelatin to form complexes with stronger surface activity. Consequently, charge density of protein and polysaccharide increased together, which is propitious to rearrange the soluble complexes into the capsule wall. It was reported that was formed during the formation of. Compared with gelatin/Arabic gum microcapsules formed in the presence of SDS [43] which owns the capsule wall of porous microstructure.

3.5. Microencapsulation Efficiency (EE) and Encapsulation Yield (EY)

Total oil (TO) contents of MFO1 and MFO2 were calculated to be 10.86 ± 0.33% and 11.92 ± 0.25%, respectively (Table 1). MFO1 had surface oil (SO) content of 2.69 ± 0.89% which was higher than the 1.99 ± 0.67% measured in MSO. These results are comparable to Drusch [52] and J.D Estrada [53] results which indicated that depending on the extraction method used, oil load and spray drying conditions, the SO of fish oil

microcapsules ranges from 0.99 ± 0.03 to 13.5 %. SO content is an important attribute evaluated in spray dried encapsulated oil microcapsules as it influences oxidative stability, wettability, flowability and color [54].

Table 1. Physicochemical characteristics of microencapsulated fish oil one shell (MFO1) and microencapsulated fish oil multishells (MFO2).

Item	Fish oil	
	MFO1	MFO2
PV, mEq/Kg of oil	2.98 ± 0.12	2.09 ± 0.05
AV	3.92 ± 0.04	2.67 ± 0.13
TOTOX	9.88 ± 0.06	6.87 ± 0.13
FFA, %	0.84 ± 0.03%	0.78 ± 0.02%
Moisture, %	4.8 ± 1.6	6.0 ± 1.9
Water activity	0.13 ± 0.01	0.14 ± 0.02
Hygroscopicity, %	30 ± 0.6	28.9 ± 0.5
Carr's Index, %	1.44 ± 0.2	1.42 ± 0.11
Hausner Ratio	30.02 ± 1.6	29.04 ±5.97
Total oil, %	10.86 ± 0.33%	11.92 ± 0.25%
Surface oil, %	2.69 ± 0.89%	1.99 ± 0.67%
Microencapsulation efficiency, %	75.2 ± 0.73%	82.81 ± 0.618%
Encapsulation yield, %	72.68 ± 0.51%	76.86 ± 0.46%
Color L*	73.07 ± 0.02	72.01 ± 0.005
Color a*	6.35 ± 0.02	4.84 ± 0.02
Color b*	8.76 ± 0.01	12.8 ± 0.01

The EE for the encapsulation process of MFO1 and MFO2 were 75.2 ± 0.73% and 82.81 ± 0.618%, respectively (Table 1). It reflects not only the non-encapsulated oil present on the surface of microcapsules but also the proportion of oil extracted from near the surface of the capsules [55]. A number of studies comparing the EE [18, 52, 56-58] of different microencapsulating agents are readily available. The EY calculated for our study were 72.68 ± 0.51% and 76.86 ± 0.46% for MFO1 and MFO2, respectively (Table 1). Weigang Li [21] shows the yield of the complex coacervation as a function of the SDS concentration. It is found that the yield of the system without SDS is very lowand the addition of SDS enhances the yield remarkably.

3.6. Flowing properties

The flowing properties for fish oil microcapsules are listed in Table 1. According to the Turchiuli's classification [33] for powder flowability, microcapsules prepared by spray- and freeze-drying had poor or very poor handling properties. The microcapsules prepared in this study were very compressible compared to other oil

microcapsules prepared by spray drying as reported in the literature [33, 59, 60]. The higher Hausner ratio means that the powder is more cohesive and less able to flow freely. The same result was observed for microcapsules produced by spray or freeze drying, despite the decrease of the Hausner ratio and Carr index [59]. Fuchs and Turchiuli [59]reported similar results of poor flowability for oil microencapsulated. The bulk densities found in this study were typical of encapsulated powders [60].

3.7. Peroxide Value, Anisidine Value, Total Oxidation, Free Fatty Acids and Color of microcapsules

Peroxide Value (PV) is an indicator of initial lipid oxidation. The PVs (Table 1.) of MFO1 and MFO2 were 2.98 ± 0.12 and 2.09 ± 0.05 meq/ kg oil, respectively. According to Gracey [61], oil with a PV below 5 meq/ kg can be considered fresh oil or one in which hydroperoxides have degraded into secondary oxidation products, like ketones and aldehydes. The secondary oxidation products measured as AV (Table 1.) were determined to be 3.92 ± 0.04 and 2.67 ± 0.13 for MFO1 and MFO2 respectively. The Council for Responsible Nutrition (2006) set a fish oil quality standard of an AV less than or equal to 20. According to equation (9), MFO1 (9.88 ± 0.06) had a higher TOTOX value than MFO2 (6.87 ± 0.13). The FFA values for MFO1 and MFO2 (Table 1) were well below 3% and were estimated to be 0.84 ± 0.03% and 0.78 ± 0.02%, respectively. The FFA content of MFO may be influenced by hydrolysis promoted by higher moisture content (Table 1) in MFO or in the encapsulated PFO. FFA content greater than 3% is considered inedible [61]. Yin and Sathivel [62] concluded that an increase in FFA content during storage may be influenced by hydrolysis promoted by the initial moisture content of the oil.

The color L*, a* and b* values are reported in Table 2. MMO had a lighter color than PAM and MSO. Results for a* indicated that MSO had a redder color compared to PAM and MMO. MMO and MSO had significantly higher b* values (more yellowness) which may be attributed to the presence of FO around the microcapsules.

3.8. Differential scanning calorimetry

DSC was used for studying thermal transitions occurring in the course of heating under an inert atmosphere (figure 6). According to the integral procedural decomposition temperature (IPDT) values calculated based on the TGA Thermograms by Zohuriaan [63]. The gradual weight loss, not more than 20%, of microcapsules is found below the degradation temperature, due to the desorptions of small molecules such as H_2O as well as the release of core material [21]. The transitions associated with loss of water (2–10 wt%) correspond to the hydrophilic nature of functional groups of the respective polymer. Furthermore, the particle size of the samples affects this transition to some extent. On the other hand, no glass transition temperature (T_g) was recorded. The reason may be attributed to interference of the T_g transition by the moisture endothermic peak. The glass transitions may also lie at temperatures lower than the starting temperature of the DSC analysis, i.e. 27 °C.[63].

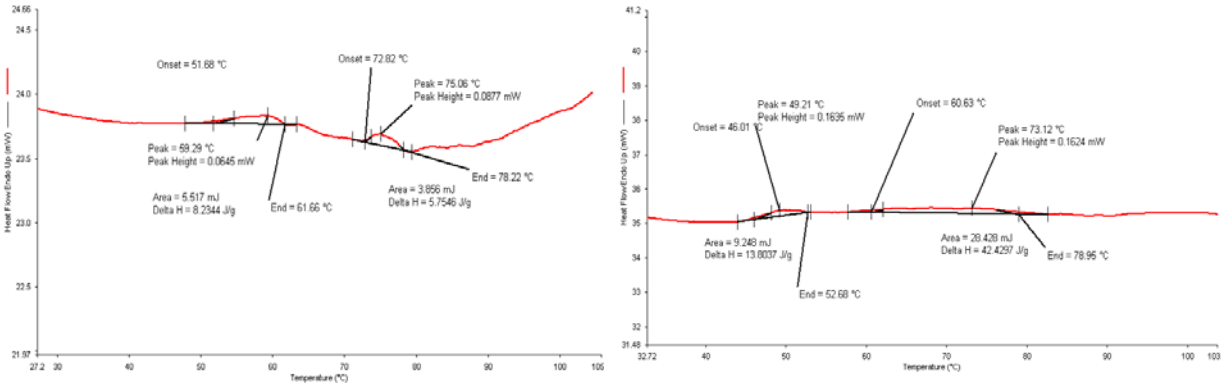

Fig. 6. Differential Scanning electron (a) microcapsules of Gelatin and NaCMC (b) Multi shells

3.9. Infrared Spectrum analysis of gums and microcapsules

The infrared spectrums of gelatin, sodium CMC, sodium polyphosphate and fish oil loaded microcapsule were shown in Figure 7**.**

The peak values of particles from complex coacervation approximated those peak values of gelatin existed in products as the peak values are approximate at the wave numbers of 3407.33, 3065.76, 2963.48, 1646.95; also, sodium carboxymethylcellulose at the wave numbers of 3441.05, 2924.99, 1607.80, 1510.79, 1111.76, 1045.50, respectively, showing sodium CMC present in capsules. We can observe the same with multishells microcapsules; Sodium polyphosphate existed in microcapsules as the peak values are approximate at the wave numbers of 1576, 1211.84, 1167.67, 735.94, 606.33. No generation of new chemical bond evidenced by no specific peak value found between spectrums of microcapsules and wall materials, further confirms the formation of complexes promoted by physical interaction such as electrostatic interaction rather than chemical reactions [64, 65] By comparison, it is verified that the interaction between gelatin and gum arabic was based on electrostatic force.

3.10. Oxidation stability of microcapsules

The most common mechanism by which the fish oil oxidizes is by autooxidation. The autooxidation reaction proceeds through a free radical mechanism involving three phases: initiation, propagation and termination [66, 67].

3.10.1. Effect of light on stability of microcapsules.

Figure 8.1 shows that after 28 days storage with light, the retention rates of products were 79.4 % for MFO2 and 71% for MFO1; also we know that direct initiation of fish oil oxidation by light requires either direct deposition to sufficient energy to break covalent bonds or transformation of light energy to chemical energy that can catalyze the reaction. Although ultraviolet light is thermodynamically capable of producing L• radicals directly

in lipids, the process is not a competitive reaction. The main reason is that the principal light-absorbing groups of lipids are double bonds, peroxide O-O bonds, and carbonyls; the last two are most important. The primary mechanism by which ultraviolet radiation initiates lipid oxidation is actually indirect, mediated through homolytic scission of any preformed hydroperoxides to generate the true initiators—LO•□,HO• and RO•□that abstract hydrogens from lipid molecules and form the ab initio L•. They can also damage fatty cellular membranes, further increasing health dangers [68].

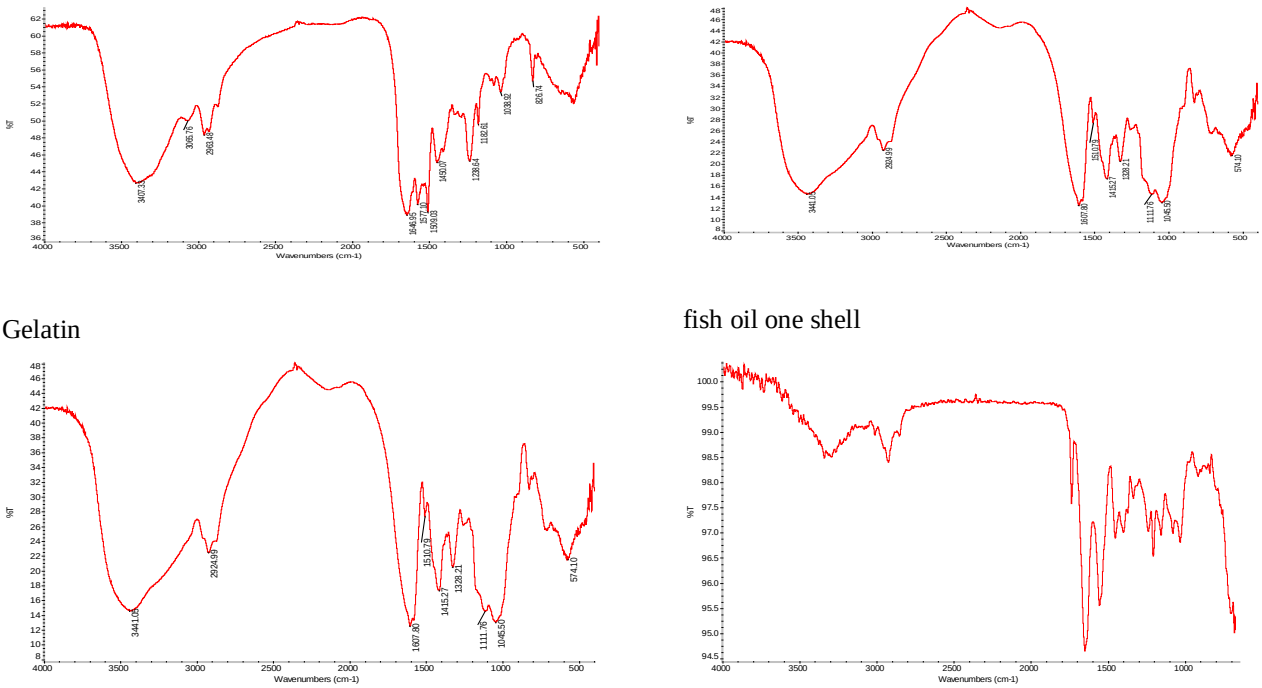

Fig. 7. Infrared spectroscopy analysis.

3.10.2. Effect of oxygen on stability of microcapsules

Figure 8.2 shows that after 28 days storage in aerobic medium, the retention rates of products were 76.5 % for MFO2 and 69.4% for MFO1. We know that since fish oil contains a high amount of pro-oxidative haem proteins as well as free ions, the initiation step could also take place easily. In the presence of atmospheric oxygen, the propagation more likely occurred [69]. However, only very small amounts of oxygen are necessary to initiate the autoxidation process. It is impossible to predict the minimum amount of oxygen necessary to give rise to detectable oxidation products in these complex heterogeneous matrices. In these powders, oxidation is heterogeneous since some triglyceride molecules are probably much more exposed to the occluded air than others. Therefore, although the amount of oxygen available is not high, a part of the encapsulated oil can be in

direct contact with the occluded air and develop detectable off-flavor, indicating the end of shelf life. However, when the powder sample is extracted and the total encapsulated oil analyzed for chemical indicators of oxidation, chemical changes may not be detected because of the high proportion of unoxidized oil [70]. The effects of PUFAs can also differ depending on in vivo and in vitro conditions [71, 72]

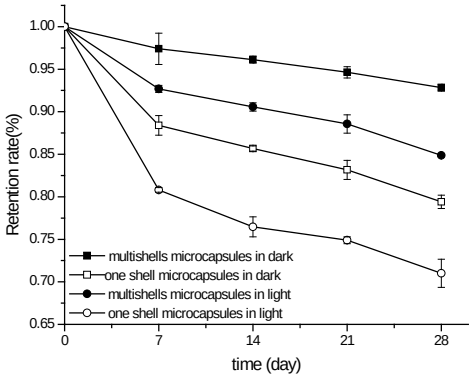

Fig. 8.1. Effect of light on stability of microcapsules

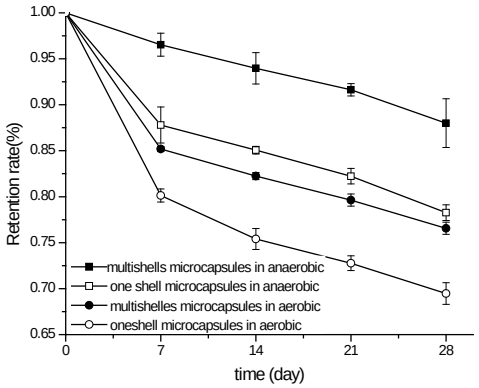

Fig. 8.2. Effect of oxygen on stability of microcapsules

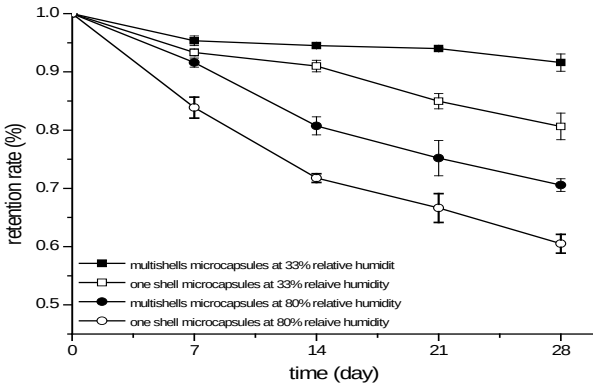

Fig. 8.3. Effect of relative humidity on stability of microcapsules

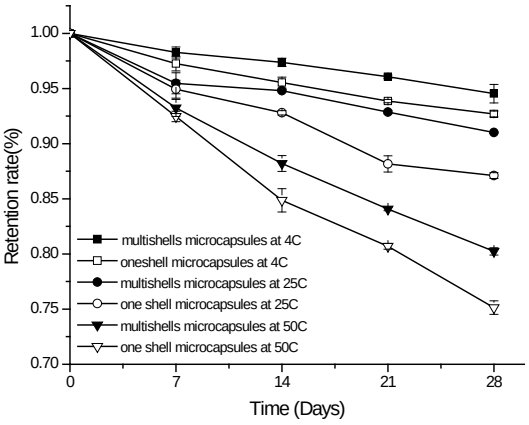

Fig. 8.4. Effect of temperature on stability of microcapsules

3.10.3. Effect of relative humidity on stability of microcapsules

Relative humidity is taken as significant factor influencing product storage. Gelatin, sodium CMC and sodium polyphosphate have strong hygroscopicity, when placed in high humility environment, promoting the increase of water content in products and the consequent gradual loss of fish oil and structural collapse of capsules. The results (Fig. 8.3) showed that after 28 days storage with 33% relative humidity, the retention rates of products

were 91.59 % for MFO2 and 80.63 for MFO1.When kept in 80% relative humility for 28 days, the retention rates were 70.56 % and 60.5% respectively. It demonstrated that the products should be preserved in low relative humidity conditions.

3.10.4. Effect of temperature on stability of microcapsules

Figure 8.4 shows that the increasing of temperature has a bad effect on fish oil microcapsules stability during storage. This figure also shows that after 28 days storage with light, the retention rate of products decreased gradually. Fish oil oxidation, assessed by hydroperoxides, headspeace aldehydes, and malondialdehyde (MDA), was found significant in microcapsules. These results are similar with those obtained by Davis and Michalski [68, 73]. It's clear that High temperatures have sufficient energy to break covalent C-C or C-H bonds in the acyl backbone to form a variety of lipid alkyl radicals, which then start the radical chains of oxidation. Moderate temperatures have lower energy, so act primarily by breaking O-O bonds in traces of ROOH or LOOH preformed by other reactions, particularly metals, lipoxygenase, or photosensitizers. The RO•, LO•, and •OH thus generated abstract hydrogens from neighboring lipids to form L• and initiate radical chains.

3.11. Fatty acid composition of fish oil

According to the GC results of the fatty acid composition of lipids extracted, polyunsaturated fatty acid (PUFA) in MFO accounted for 78.95% of the fatty acids detected, and EPA and DHA were the predominant fatty acids, accounting for 83.85% of the total PUFA content.. The fatty acid composition of the fish oil was in agreement with the profiles previously reported by [53]. The changes in fatty acid composition over storage are the subject of another report. There was no change in the ω-3 fatty acid content due to the encapsulation process for spray-dried particles. But, the content of ω-3 fatty acid decreased for freeze-dried particles as the surface oil content was high.

4. Conclusion

Complex coacervates for carrying and protecting EPA and DHA in fish oil were designed to yield a free flowing powder that acted to inhibit the production of primary and secondary oxidative products during 4 weeks of storage at room temperature, relative to the bulk oil. Using the Response surface Model, it was demonstrated that particle yield and microencapsulation efficiency were affected by the ratio of core and wall material, the pH and the concentration of surfactant in the process. Capsules formed by complex coacervation had sufficient stability through electrostatic attraction to maintain their structure. Furthermore, the statistical model in this study predicted successfully the microencapsulation efficiency. On the other hand, despite the high particle yield, freeze-dried microcapsules showed low microencapsulation efficiency. The use of this encapsulation design could lead to increased utilization of fish oil in aqueous food systems, so as to contribute to the health and well-being of consumers.

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